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PROPERTIES OF ENZYMES VII. PURIFICATION AND PROPERTIES OF HUMAN PLACENTAL SUPEROXIDE DISMUTASE, AND TOTAL PEROXIDASE ACTIVITIES IN NORMAL AND PATHOLOGICAL CASES

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The results achieved in connection with the purification and properties of human placental superoxide dismutase are reviewed. The rough molecular weight of the purified enzyme was determined. After the hydrolysis of enzyme protein **2** its quantitative aminoacid composition was determined and compared with data for other human superoxide dismutases. The changes in the total superoxide dismutase and total peroxidase were brought into correlation with the age of the placenta. A certain biological periodicity can be observed in the changes of activity of the enzymes in normal placentae.

As a continuation of our earlier work (PHAM VAN HIEN, 1974; MATKOVICS, 1975), the present publication deals with the determination of the molecular weight and aminoacid composition of purified human placental superoxide dismutase (SOD; EC 1.15. 1.1.) and with the changes observed in the eznyme under normal and pathological conditions, these latter being supplemented with the changes in human placental peroxidase (HPP; EC 1.11. 1.7.) under parallel conditions.

Materials and Methods

The preparation and quantitative determination of SOD were detailed previously (PHAM VAN HIEN et al., 1974). After fractionation, precipitation, purification on a Sephadex G—75 column and concentration with Molselect G—25 (Reanal, Budapest), SOD with an activity of 2350 μmole. $min.^{-1}$ mg^{-1} was obtained; this was preserved in freeze-dried form for use in further examinations.

The quantitative determination of SOD was most often performed with the adrenochrome method (MISRA et al., 1972).

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In the comparative studies the placentae were pretreated to remove haemoglobin (PHAM VAN HIEN et al., 1974), and the tissue was then homogenized in cold 155 mmole/l NaCl solution (5 ml solution per 1 g wet tissue) in a Potter glass homogenizer (PHAM VAN HIEN et al., 1974).

The supernatant obtained in the course of the preparations after ethanol-chloroform (5:1) precipitation and centrifugation was used for the determination of SOD (KEELE et al., 1971).

For the peroxidase measurements, 1 g wet placental tissue, washed free of haemoglobin, was homogenized under the above-mentioned conditions, and the enzyme was determined on various aliquots of the homogenizate after adjusment of the pH to 7.1 with phosphate buffer.

The quantitative measurement of peroxidase was carried out at 470 nm with a Spektromom 360 (MOM, Budapest) photometer, using the guaiacol method (COLOWICK et al., 1955); a total volume of 3 ml was taken and 1 cm cells were used.

(The data determined for the enzymes always refer to 1 g wet tissue.)

The normal human placenta weights reported by KLOSTERMAN et al. (1954) were used to calculate the average values from the 24th week on (see Fig. 1. and Table 1).

In the purification of the SOD the protein content of the fractions was determined spectrophotometrically at 280 nm or by the LOWRY method (LOWRY et al., 1951), using bovine albumin to prepare the standard curve.

A Spektronom 301 (MOM, Budapest) spectrophotometer was employed for measurements in the UV region.

The absorption spectrum of the pure placental SOD was recorded in a 50 mmole/l potassium phosphate buffer (pH 7.8) at a protein concentration of 0.12 g/ml ,with a Unicam SP—800 spectrophotometer (Fig. 3).

Molecular weight determinations were performed on 20×20 cm plates coated with 0.6 mm layers of Sephadex G—100 superfine gel. 155 mmole-l NaCl solution was used for running in an ascending system, in a specially-designed apparatus (Pharmacia AG, Uppsala, Sweden) (Pharmacia booklet, 1971). The enzymes and comparison materials were dissolved in distilled water, dropped onto the plates, and subjected to running for about 4 h. The anzyme spots were transferred from the layer onto Whatman MN 214 paper with the replication technique (RADOLA ,1968); on the paper they were stained with 2 g/l (2.86 mmole/l) bromocresul green solution in alcohol-acetic acid (955:5), and fixed in ammonia vapour (Fig. 4).

The freeze-dried enzyme was used for the quantitative determination of the SOD protein aminoacids. Hydrolysis was achieved with 6 mole-l HCl for 22 h at 120 °C. The hydrolyzate was evaporated to dryness, and an amount equivalent to 0,1 mg protein was transferred to the column.

A Bio-Cal BC—200 aminoacid analyzer (Bio-Cal, Richmond, California, USA) was used, with a 52.0×0.9 cm column packed with Aminex A—6 (12—15 m) spherical resin (Bio-Rad Labs., Richmond California, USA). (The one-column technique was used.) The resin column was maintained at 52—53 °C throughout the analysis (Fig. 5, Tabl. 2). (The data in the Table give the number of aminoacids per protein molecule.)

The human placentae were obtained from the Department of Obstetrics and Gynaecology, University Medical School, Szeged. They originated in part from normal births, premature births and artificial abortions, or from interruptions brought about by intraamnial hypertonic sodium chloride solution and by administration of 25 mg prostaglandin F_{2alfa} (Upjohn, USA). (The reasons for the interruptions were at times other pathologic processes, which are mentined separately.)

Results and Discussion

Figure 1 and Table 1 contain values and average values for which primarily the data of KLOSTERMAN et al. (1954) were used.

Table 1

Duration of pregnancy (in weeks)	Weight of child (in g) (Only the minimum and maximum values reported in the literature are given)	Weight of placenta (in g)	Average weight of placenta (in g)*	Blood-free weight of placenta (in g)
8	4	8	8	
12	14—35	33	33	
16	55—150	70	70	
20	250—316	120	120	
24	600—672	195—258	226	
28	1000—1200	275—309	292	
32	1500—2115	365—483	424	
36	2200—2783	470—536	503	306
40	3000—3405	429—600	414	347

* For calculations the average values of placentae were always used.

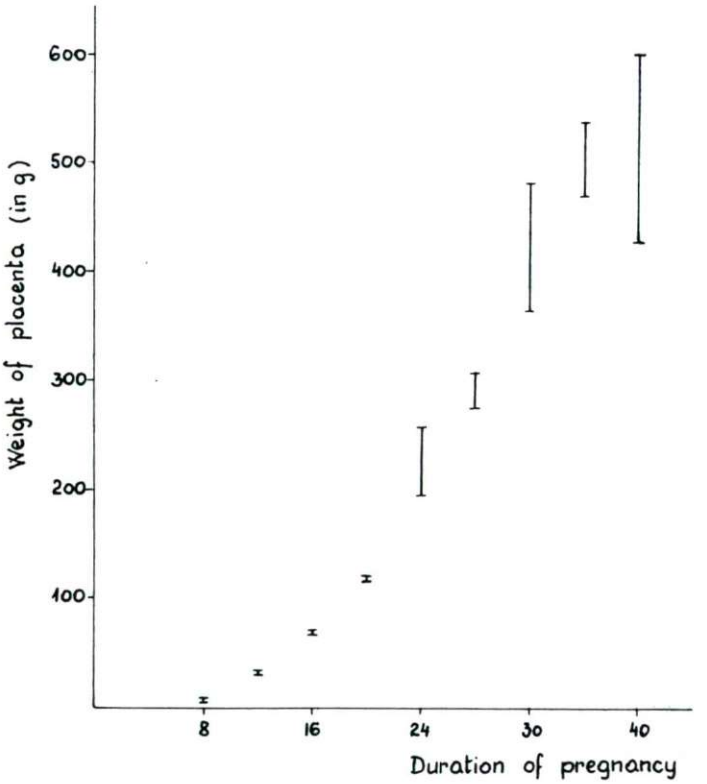


Fig. 1. Literature values illustrating average weight of placenta as a function of duration of pregnancy (KLOOSTERMAN, 1954).

Figure 2, similarly taken from the literature, is designed to show components of the full-term placenta (GARROW, 1970). Naturally, other references too could be mentioned in this respect (GELLÉN, 1969; HAMILTON, 1970).

The other Figures originate from our own experimental work. Figure 3 presents the absorption spectrum of purified human placental SOD (the details of the spectrum-recording are given in the "Materials and Methods" section).

Figure 4 illustrates the comparative SOD molecular weight values obtained with the replica technique. The comparison was performed with human and bovine erythrocyte SOD of known molecular weight. In addition, the SOD of unknown weight prepared from the full-term placenta was also depicted graphically. (Our molecular weight determinations can be said to be approximate only.)

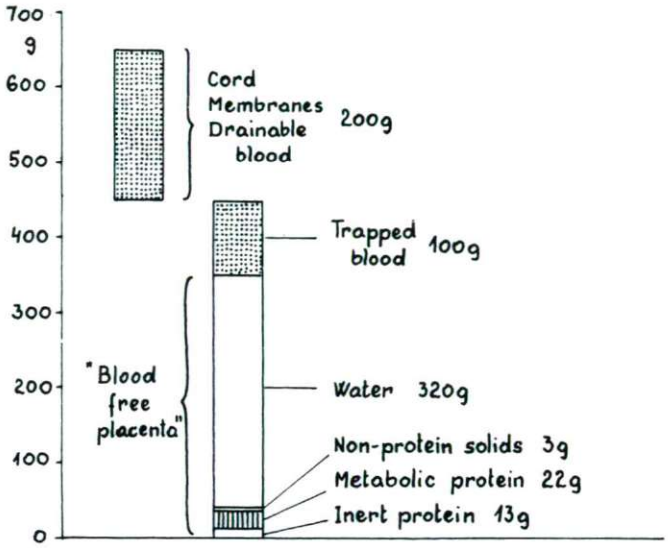


Fig. 2. Literature column-graph illustrating the main components of a full-term placenta (GARROW, 1970).

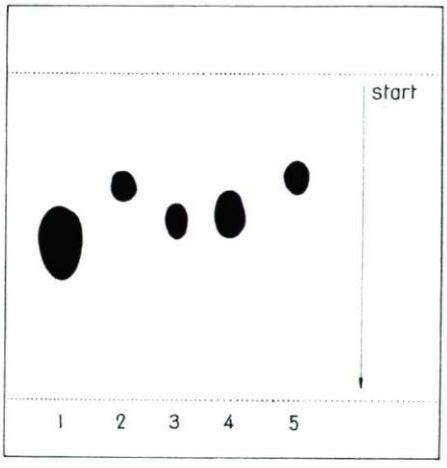


Fig. 3. UV absorption spectrum of human placental superoxide dismutase.

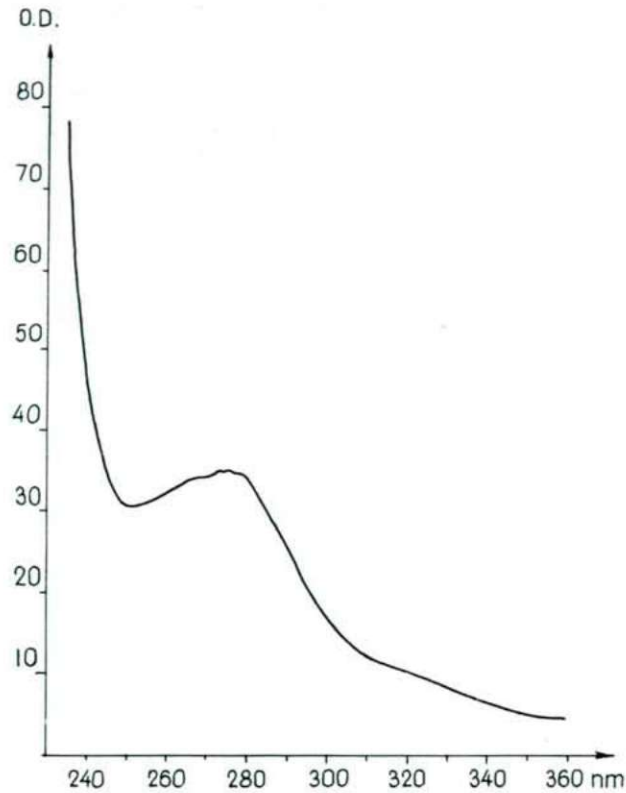


Fig. 4. Determination of molecular weight of human placental superoxide dismutase by replica techniquw

1. Pea SOD (M.W. 31,500)
2. Bovine erythrocyte SOD (M.W. 32,600)
3. Human placental SOD
4. Human placental SOD
5. Human erythrocyte SOD (M.W. 33,600)

Figure 5. presents the recorded aminoacid qualitative and quantitative values. These correspond to the data in Table 2, where, however, the quantitative data for the aminoacids were calculated via the molecular weights determined by ourselves. These permit a good comparison as regards the quantitative values for the similar human and animal (vertebrate) SOD-s

In the following three Figures (Fig. 6—8) a comparison is made of the SOD values of placentae of different ages, calculated on the total weight. It can be seen that the relative and absolute amounts of SOD increase during the development of the placenta. The Figures clearly reveal the repid rise in the SOD values of placentae developing under normal conditions. (This may be related with the protective

Table 2

Aminoacidis	Bovine erythrocytes (Kovács, 1974)	Human erythrocytes (Mccord, 1971)	Normal human placentae
	M. W. 32,600	33,600	32,000
Lys	22	22	22
His	16	16	16
Arg	7	10	5
Asp	36	35	35
Thr	16	26	19
Ser	20	20	21
Glu	26	24	50
Pro	10	14	29
Gly	50	50	23
Ala	20	21	21
Val	28	28	26
Met	0	0	3
Ile	16	17	8
Leu	17	20	25
Tyr	0	2	5
Phe	8	10	12
Trp	2	0	0
Cys/2	7	6*	2

* from Hartz, 1972.

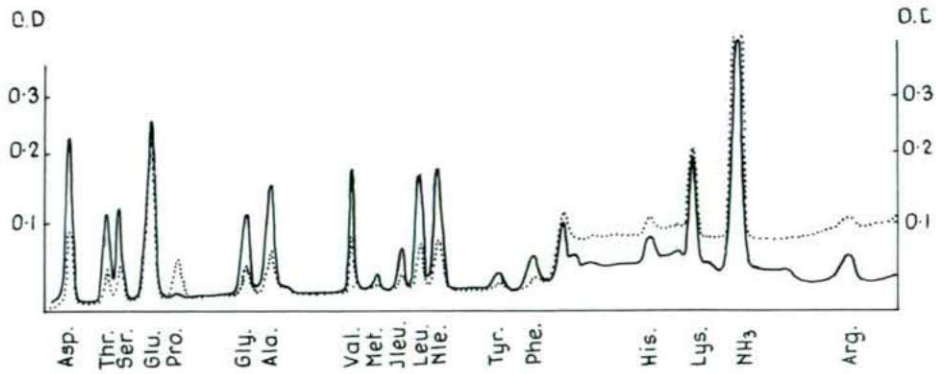


Fig. 5. Quantitative aminoacid chromatogram for human placental SOD hydrolyzate

effect of SOD, much mentioned in the literature, which tends towards the elimination of the O^{-}_{2} anion (Fridovich, 1972). (See mainly Fig. 6)

If the normal development is disturbed by anything at all, or if abortion is initiated with intraamnial hypertonic sodium chloride, or prostaglandin, for instance, the measured SOD value falls considerably. Our findings refer particularly to those cases when foetal necrosis and other pathological processes (mola hydatidosa, etc). necessitate induced abortion. (Mainly sthe data of Figure 7. are of such origin.)

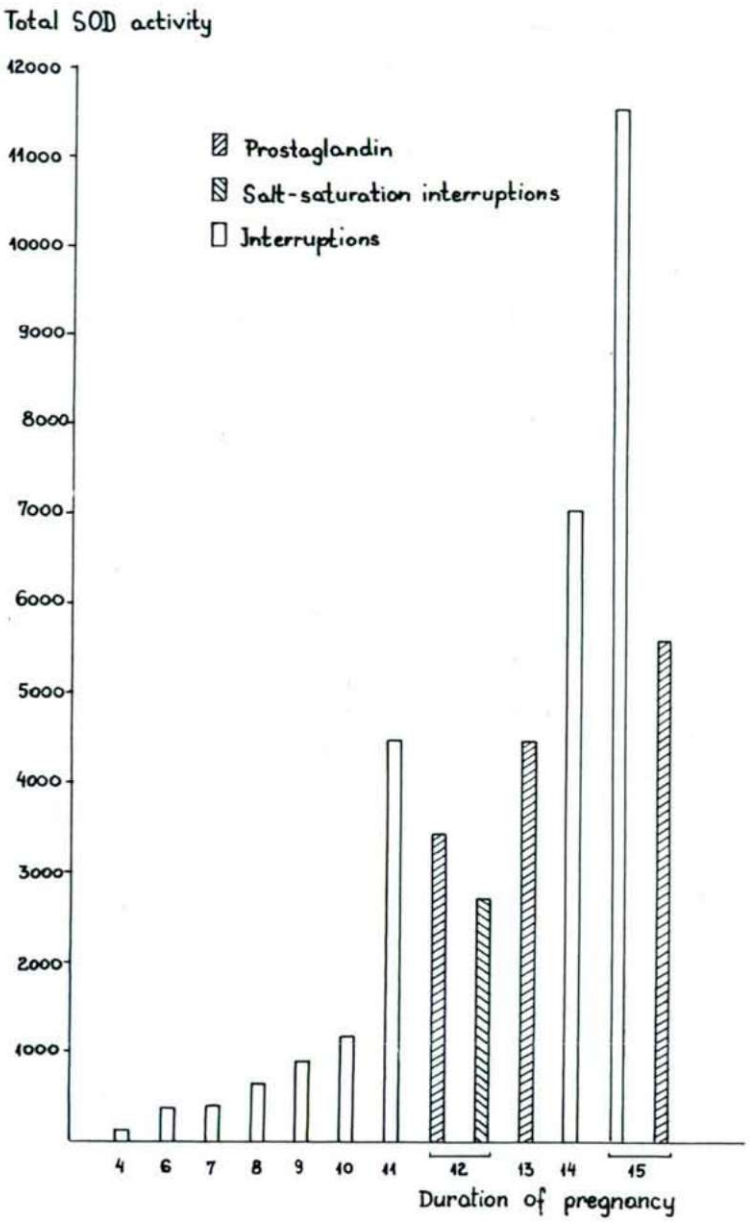


Fig. 6. Variation of total SOD activity of 4—15-week placentae. (Material originating from artificial, prostaglandin and salt-saturation abortions.)

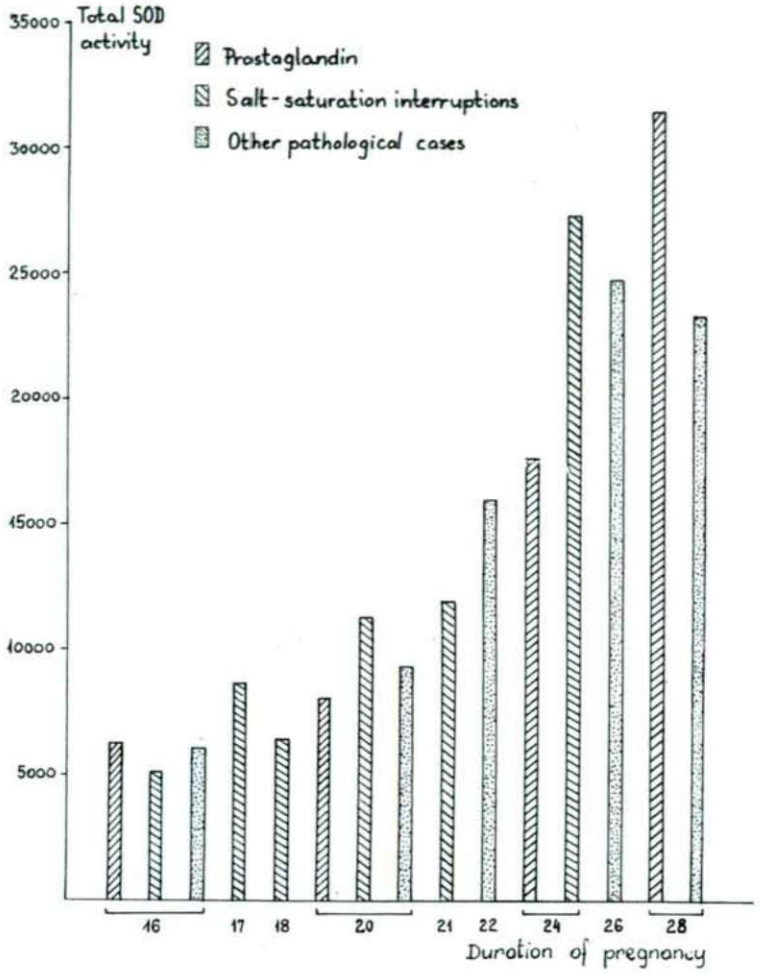


Fig. 7. Variation of total SOD activity of 16—28-week placentae. (Material originating from prostaglandin, salt-saturation and other pathological interruptions.)

(The SOD activity values in the Figures were obtained by taking the SOD values for 1 g wet placenta after the preparation detailed in the "Materials and Methods" section and multiplying by the placental weight data in Table 1).

In Figure 8 the SOD values of the full-term placentae are compared with the SOD values of placentae originating from premature deliveries.

Figure 9. compares the total SOD and total peroxidase values for placentae from artifical interruptions (4th—15th week) and full-term placentae (40th—41st week).

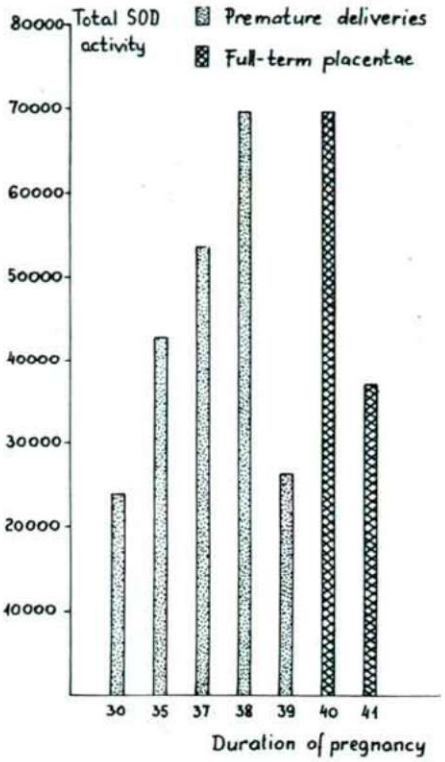


Fig. 8. Variation of total SOD activity of 30—41-week full-term placentae and placentae from premature deliveries.

Figures 10. and 11 present similar enzyme data for 12—28-week placentae. Figure 10. shows separately the total enzyme values for the prostaglandin-initiated interruptions, and Figure 11 those for the interruptions induced with hypertonic solution.

Figure 12. was obtained by dividing the total SOD value by the peroxidase value; thus, the ratio of the peroxide producing and decomposing enzymes could be characterized by values in the range 0.1—0.5 SOD produces H_2O_2 by the reaction:

$$2\,O^-_2 + 2\,H^+ \rightarrow H_2O_2 + O_2$$

while HPP decomposes the H_2O_2:

$$XH_2 + H_2O_2 \rightarrow 2\;H_2O + X_{oxide}$$

The radicals produced in the reactions can participate in many processes, of course, and may result in the formation of useful or harmful metabolites.

Figure 13. shows the reciprocal peroxide metabolism index. In this case the total HPP values were divided by the total SOD values. The values in Figure 13.

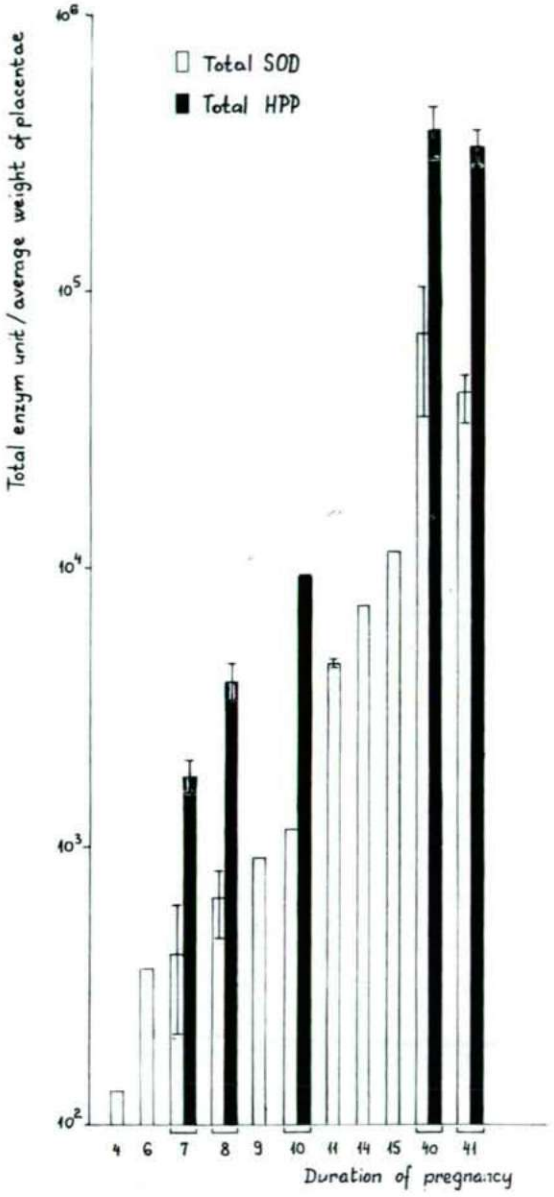
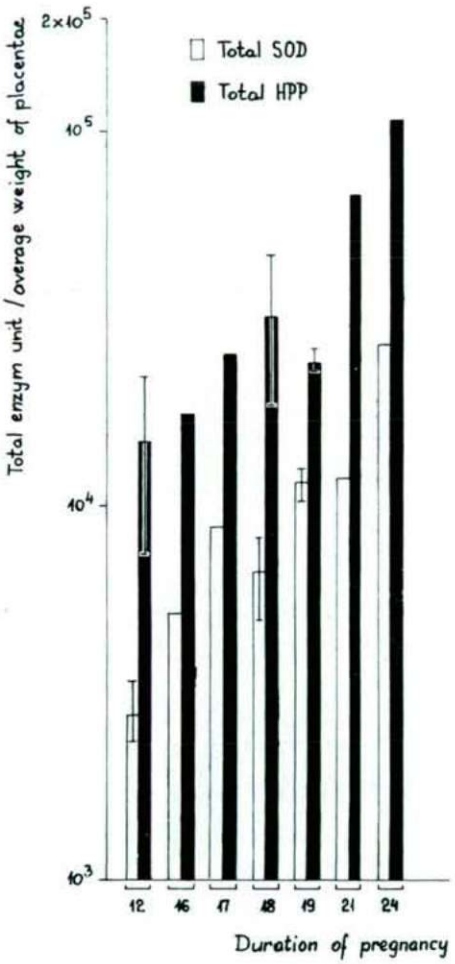


Fig. 9. Comparison of total peroxidase and total SOD activities in artificial interruption and full-term placentae.



F.g. 10. Comparison of total peroxidase and total SOD activities in material from prostaglandin interruptions.

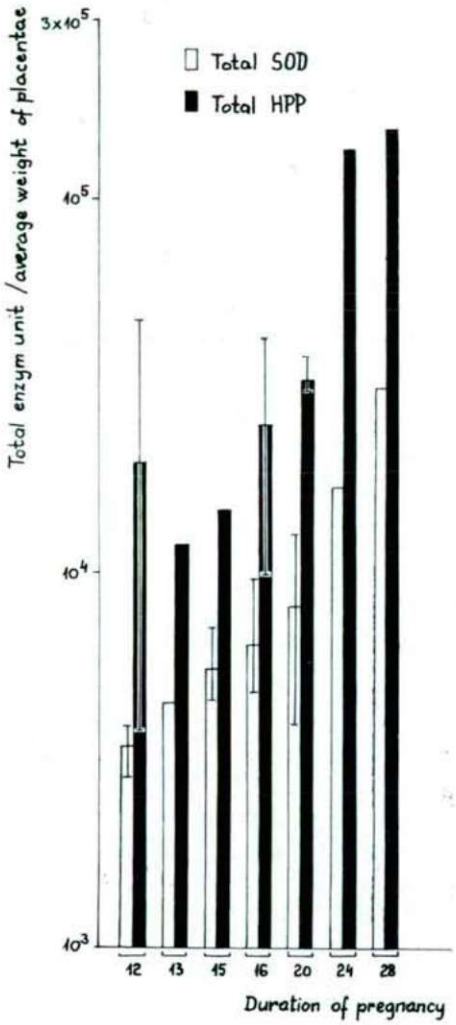


Fig. 11. Comparison of total peroxidase and total SOD activities in material from saturated-salt solution iniciated interruption cases.

lie between roughly 2 and 12; this is important perhaps from the aspect that the values are spread out more.

Both column-graphs display a certain harmonic fluctuation with regard to the two enzymes of the peroxide metabolism, reflecting a characteristic biological rhythm.

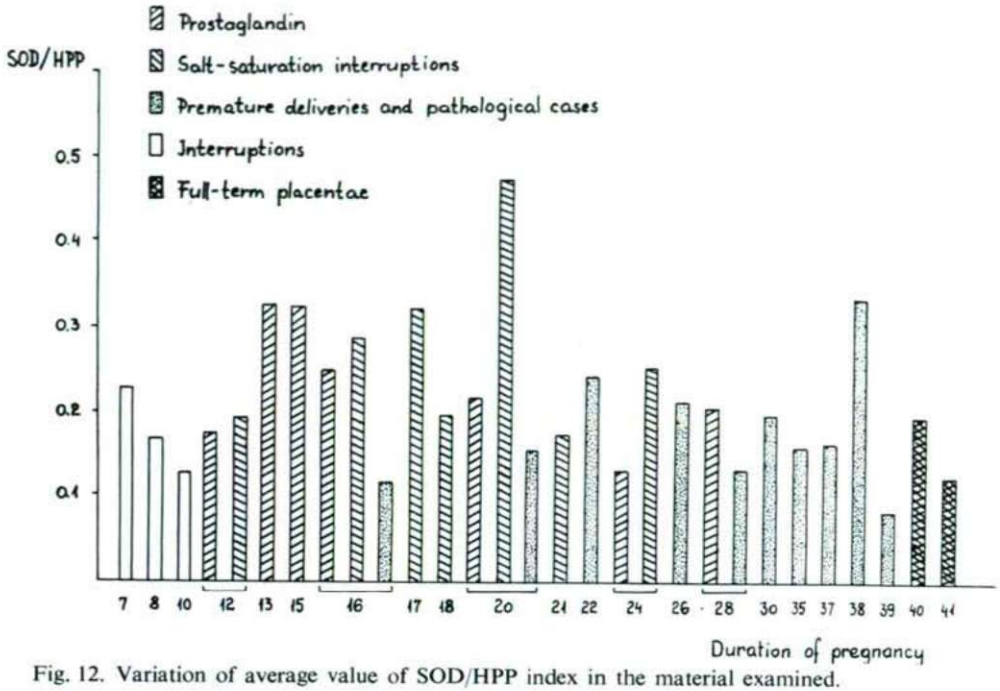


Fig. 12. Variation of average value of SOD/HPP index in the material examined.

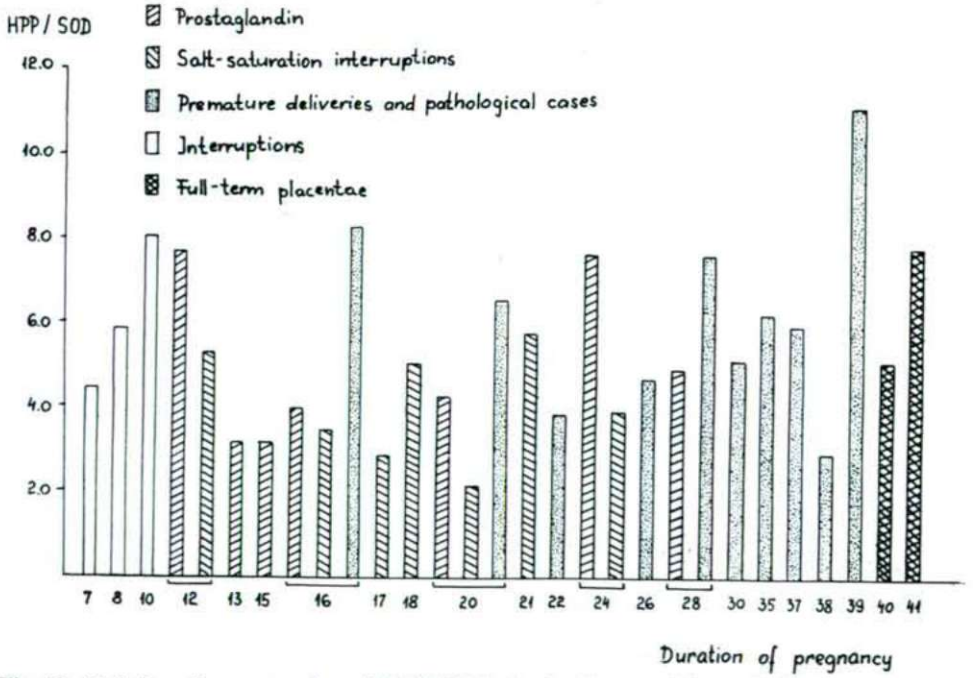


Fig. 13. Variation of average value of HPP/SOD index in the material examined.

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